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Human Viral Respiratory Disease

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EFFECTS OF POLLUTANTS ON HUMAN VIRAL RESPIRATORY DISEASE

by

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Abstract

Many epidemiologic studies have shown excessive respiratory disease morbidity in areas of high atmospheric pollution. This study was designed to develop and characterize an animal model and investigate the possible interactive effects of infection and particulate air pollutants using small laboratory animals. Models of human parainfluenza virus type 3 disease were established by aerosol inoculation of hamsters and cotton rats. The temporal course of the following were examined: lung virus titers; pulmonary histopathology; alveolar macrophage function; changes in pulmonary mechanics; serum antibody development and upper respiratory tract histopathology. Animals were exposed acutely (2 hours) to ammonium nitrate or lead oxide respirable aerosols before or following viral inoculation. Exposures ranging from 59-66 mg/m³ but not 0.76 mg/m³ of the nitrate resulted in a one-day extension of viral replication and concomitant retardation of peribronchial lymphocytic infiltration. Lead oxide exposures at levels greater than 2,350 µgm/M³ increased lung virus titers and serum antibody titers. The models developed in these studies may be useful for future work on chronic exposure to the same or other pollutants and on the pathogenesis of virus/pollutant interactions.

INTRODUCTION

The observed excess of acute respiratory disease morbidity in areas of high atmospheric pollution lacks a clear explanation at present. This excess cannot be explained by differences in the infecting agent since it is known that the same infectious agents produce infections in both urban and rural settings. One possible explanation for this excess mortality is that pollutants may interact in some way with either agent or host to the disadvantage of the host. Since a complete study of the problem is not feasible in the normal human population, animal models can be used to obtain detailed information which can then suggest hypotheses and limited studies that could be accomplished in people.

Since most acute respiratory disease in humans is caused by virus infections, small laboratory animals models of the common types of infections would be useful to assess the added effects of inhaled toxicants. This report describes studies in which hamster and cotton rat models of parainfluenza virus type 3 (PV3) were characterized and used for initial evaluation of the effects of ammonium nitrate and lead oxide. The development of methods, pollutant exposure results and conclusions will be presented.

MODEL DEVELOPMENT

Animal Infection

Syrian hamsters (Mesocricetus auratus) were purchased from commercial sources and maintained in the facilities of the Division of Laboratory Animal Medicine of the School of Medicine. Cotton rats (Sigmodon hispidus) were produced from an on-site breeding colony using stock obtained originally from the National Institutes of Health. The colony is screened periodically for infections by Sendai virus (complement fixation serology), Mycoplasma pulmonis (culture) and other rodent pathogens (histology of lungs, Harderion glands and middle ear cavities).

The usefulness of the hamster as a model of human parainfluenza virus type 3 infection was described by Buthala and Sorret (1). Since this model had been established and evaluated in our laboratories for other studies (2) it was used initially in the current work. Prototype virus strain HA-1 was obtained from the National Institutes of Health (Reference Reagent Catalog No. V-323-002-020) and was propagated in Hep 2 cell cultures. Stock virus and cells were quality controlled for mycoplasma contamination using the fluorochrome stain method applied to test cultures in known-negative Vero cells (3).

An important early goal was the establishment of aerosol inoculation capability in our laboratories. The equipment used was that described by Hu, et al (4) which had been developed to inoculate hamsters with Mycoplasma pneumoniae. The device allows

"nose-only" inhalation exposure of 8 animals held in individual ports. A glass nebulizer generates the aerosol, and passage through a glass drying column yields a product with mainly inhalable particles. In evaluation of the generator for use with PV3, it was found that minimal foaming was obtained if virus was grown in a medium with only 1% fetal bovine serum. Air flow to the drying column had to be reduced to prevent loss of virus viability. Reproducible infection of hamsters and maximal virus yield from lung tissue was achieved by delivery of $10^{7.9}$ TCID₅₀ of virus into the chamber over a 20-30 minute period.

Following standardization of the aerosol exposure conditions, it was possible to study the time course of the experimental infection. No virus could be found in the lungs 30 minutes after the aerosol exposure. On day 1, titers increased to $10^{5.8}$ TCID₅₀/gm lung, peaked at $10^{6.8}$ on day 2 then declined gradually with disappearance by day 6 or 7. Pathologic changes in the lung were most marked on day 5 or 6, and consisted of interstitial and peribronchial lymphocytic infiltration and marked proliferation of the bronchial epithelium. These findings were very similar to our earlier results obtained by intranasal instillation of PV3 (2). A problem arose as work continued, in that the infection and pathology on additional aerosol exposures became unpredictable. It was suspected that the hamsters were becoming infected with PV3 or a related virus (i.e., Sendai or murine parainfluenza type 1) at the suppliers and thereby were resistant to the inoculations. A survey of suppliers revealed none who would certify

freedom of their colonies from PV3 or Sendai virus.

To assess the possible magnitude of the problem of Sendai virus infection resulting in PV3 resistance, some cross-challenge experiments were done using cotton rats from our breeding colony. Groups of animals were inoculated with Sendai virus and allowed to recover (30% mortality was noted). Survivors were then exposed to varying amounts of PV3 by intranasal instillation, and the doses of virus required to infect 50% (ID_{50}) and produce pneumonia (PD_{50}) were calculated. Data are shown in Table 1. Rats previously infected with Sendai required 100-fold more PV3 to achieve an infection rate of 50% compared to controls. The amount of PV3 needed to produce pneumonia was reduced by a like amount. The effect on the ID_{50} indicates cross-reacting immunity from the prior Sendai infection, and the reduced PD_{50} is thought to reflect the same thing. It has been noted in other models that challenge infection with the same agent may exaggerate and accelerate pneumonia changes, suggesting that many of the changes seen are the histologic reflection of immune responsiveness (5). A dose-response effect for Sendai infection was shown in that prior infection with 10^5 TCID₅₀ had no effect on subsequent PV3 challenge (Table 1). Thus, freedom from Sendai virus infection is an important prerequisite for studies of PV3 in hamsters. Since this cannot be guaranteed currently by any known supplier, animals must either be bred in isolation or pre-screened serologically before use. For this reason, subsequent work was performed primarily in cotton rats from our colony.

The cotton rat is known to be susceptible to infection with Mycoplasma pneumoniae (6) and with respiratory syncytial virus (7). With evidence that it was also susceptible to human PV3, a complete study of this model was made and subsequently published (8). The course of infection and of pulmonary histopathology were very similar to those described for the hamster, both being quite similar to the natural human disease. Another advantage of the cotton rat is that they show greater immune responsiveness than hamsters; the chief disadvantage is that they are not available commercially at the present time.

Macrophage Studies

A prime defense mechanism in the lung is the alveolar macrophage. Since many inhaled intoxicants affect this "first line of defense", as can several viral respiratory diseases, evaluation of macrophage function could be a sensitive indicator of interaction between pollutants and infectious agents. Studies were undertaken of cotton rat alveolar macrophage function during the course of PV3 infection. The functional state measured was oxidative metabolism as reflected by chemiluminescence of zymosan-stimulated cells.

Bronchopulmonary cells were collected by endotracheal lavage at intervals during experimental PV3 infection. The characteristics of these cells are shown in Table 2. At each time period, cells were suspended in a luminol solution, and background light emission was measured in an ATP spectrophotometer. Zymosan was then added, and the stimulated chemiluminescence of cells

from infected animals was assessed with matched controls. By day 2 of infection chemiluminescence was decreased to 44% of control ($p = < .025$) and on day 4 to 28% ($p = < .001$). Suppression was 65% of control on day 7, and 84% on day 10, neither of these being statistically significant.

Exploration of the cause of reduced macrophage oxidative metabolism suggested that the cells were undergoing a non-proliferative infection with PV3. Macrophages cultured in vitro could not be infected with the virus. However, three-fourths of cells collected from infected animals contained viral antigen as shown with the indirect immunofluorescence method. To prove that the cells were infected, as opposed to containing phagocytosed dead virus, hemadsorption was done of cell preparations using guinea pig erythrocytes. Approximately one-fifth of the cells were hemadsorption positive, indicating expression of viral proteins on the external macrophage membrane. Attempts to verify this by electron microscopy were unsuccessful. More details on this subject are given in the attached manuscript "in preparation for publication".

Upper Respiratory Tract Models

For some studies of inhaled pollutants it may be of interest to investigate the upper respiratory tract, since considerable deposition of inspired materials takes place there. In small laboratory animals it is difficult to obtain accurate samples for quantitative study from the nasal passages, and the cartilage and bone of the skull interfere with histological studies. Some methods were developed which could be useful in future work.

For quantitative sampling of the nasal passages, the use of a 3 mm instrument cleaning brush was tested. The brush tip is identical to those used to collect specimens during bronchoscopy, and has been used in other work in our laboratories for nasal biopsies in adults and children. In either hamsters or cotton rats, insertion and twirling of the brush in the nasal passages allowed removal of an epithelial tissue sample which packs the area among the brush bristles (often 3-4 mg of material). The collected cells can then be shaken from the brush by vibration, for purposes such as virus titration, or solubilized for assays such as heavy metal quantitation.

Histologic study of the nasal passages and paranasal structures was made of PV3-infected cotton rats. At various points during experimental disease at necropsy the head was removed, freed of surface tissue and muscle and fixed in 10% neutral formalin. After fixation the skull was placed in de-calcifying solution (primarily made of HCl) followed by thorough washing in water. A sharp blade was then used to make sections across the head between the nose tip and orbits, through the orbits, and through the posterior plane containing the otic bullae; after histological processing these specimens provided frontal views of the nasal turbinates, Harderian glands and paranasal sinuses and the middle ear cavity, respectively. In PV3 infection marked nasal epithelial cell proliferation was noted, but no infiltration of the Harderian glands was seen. Cytopathic changes were present in the ciliated epithelium lining the middle ear cavity, but

there were no exudates in the lumen.

STUDIES OF PULMONARY MECHANICS IN PV3 INFECTED ANIMALS

Studies of pulmonary mechanics in young male hamsters infected with PV3 were conducted to determine the effects of the infection on lung function during the period of time when viral titers from lung homogenates and cellular reaction are greatest. One hundred gram animals were inoculated using the aerosol technique described above. Similar groups of animals were sham inoculated using viral carrier medium as the inoculum. Pulmonary mechanics were studied 5 days after inoculation using techniques previously described (9). Five separate experiments were conducted which included a total of 24 control and 24 infected hamsters. The data from these studies are in Table 3. Although the tidal volume was significantly decreased in the infected animals as compared to control, the minute ventilation was increased which resulted from an increase in respiratory frequency. There was a significant decrease in compliance in the infected hamsters when compared to control and an increase in expiratory resistance. Although inspiratory and average resistances tended to be greater in infected animals, they were not significantly different from controls. These data are consistent with findings from studies of humans with acute viral bronchiolitis and also with results anticipated after reviewing the histology of the lung from infected hamsters. Although the changes in mechanical and ventilatory parameters are small, the utilization of these techniques

in this animal model of a very common human disease should prove to be very useful.

PARTICULATE POLLUTANT EXPOSURES

A series of studies were performed in which animals were exposed acutely (2 hours) to particulate aerosols of ammonium nitrate or lead oxide before or during experimental infection with PV3. All pollutant exposures were performed and controlled by the engineers at HERL-RTP; virus exposures were done in our laboratories as described before. As a minimum study parameters in all experiments were lung histology and virus titration; additional examinations in some cases included measurement of pulmonary mechanics and viral serology. No natural mortality was observed with any of the combinations of exposures.

Ammonium Nitrate Exposures

The animals were exposed in a nose-only fashion using a Lovelace nose-only inhalation chamber (22L internal volume). All exposures were 2 hours in duration.

The aerosol was generated by nebulization of an aqueous solution of NH_4NO_3 using a Constant Output Atomizer (TSI Model 3076). The output of the generator (315 LPM) passed through a large liquid particle impactor (Anderson, Inc.) prior to being combined with dried dilution air (18.5 LPM). The aerosol then passed through a diffusion drier and a KR-85 source (Model 3012, TSI) to reduce the static charges to a normal bipolar Boltzmann distribution prior to entering the chamber.

The chamber exhaust was scrubbed using a large aqueous impinger arrangement in combination with a High Efficiency Particulate Aerosol

filter (MSA) and a charcoal filter prior to being exhausted to the atmosphere.

Mass concentrations of the aerosol were quantitated by two methods. Filter samples were taken continuously during the exposures and then analyzed either by a Nitrate Specific Ion Electrode (Orion) or an Ion Chromatograph (Dionex). The chamber was sampled at 2.15 LPM using a BGI 47 mm Teflon filter holder and 1.0 μm pore size Fluropue (polytetrafluoroethylene) filter.

Particle size distributions were quantitated by a 100 cc/min 7 stage impaction based on the design by Mercer et. al. (10) Glass filter stages were diluted with deionized water and subsequently analyzed by ion chromatography.

A group of exposures of PV3-infected cotton rats to NH_4NO_3 were completed and analyzed. Two protocols were used. In the first type of experiment, NH_4NO_3 exposure was given initially followed by PV3 inoculation on the same day. The second protocol involved PV3 infection first, followed by NH_4NO_3 exposure on day three or day five of experimental disease, with sacrifice 24 hours later when maximum virus yield would be expected (early sacrifice) or at the time of peak pulmonary histopathologic change (later sacrifice). These experiments were done with a very high-level exposure to NH_4NO_3 particles in air ranging around $60 \text{ mg}/\text{M}^3$ for two hours. One experiment was performed with a much lower level of the pollutant, $0.76 \text{ mg}/\text{M}^3$ (MMAD = $0.412 \text{ }\mu\text{m}$ $\sigma_g = 1.51$) of air for two hours.

Table 4 provides data produced by the first protocol in which NH_4NO_3 exposure preceded PV3 aerosol inoculation. Control groups were animals handled identically but exposed only to PV3 or to NH_4NO_3 aerosols. As can be seen, animals given pollutant followed

by virus had less pulmonary histopathologic change on day three of disease but more on day six than did rats given virus alone. However, less PV3 was recovered on day six from animals given NH_4NO_3 than from the infected controls. While the differences are not significant by student t-test, the effect suggested that NH_4NO_3 had an effect on both virus and host in terms of virus peak yield and prolongation of pulmonary disease.

An example of experiments in which NH_4NO_3 intercurrent exposure was given during PV3 infection is provided by Table 5. The pollutant exposure was for two hours on either day three or day five of experimental disease with subsequent sacrifice points and control groups outlined in the Table. Again, it was found that the earlier pollutant exposure diminished virus yield from the lung tissue, but that pulmonary pathologic change was enhanced later. Differences in the groups did not persist during the recovery phase, as suggested by results from a few animals held for 14 days.

The effect of lesser amounts of NH_4NO_3 is illustrated by the data in Table 6. Cotton rats were exposed to 0.76 mg/M^3 of air either the same day as PV3 inoculation or three days after infection was established. The results indicated no effect of the pollutant relative to control animals given PV3 alone. It can be concluded that either NH_4NO_3 had no effect on the experimental model, or that the indicators used to measure the effects are insufficiently sensitive to permit detection of any effects.

Studies of Pulmonary Mechanics in PV3 Infected Animals Exposed to Ammonium Nitrate

Young male hamsters were inoculated with PV3 and then allowed to breathe an atmosphere containing 988.25 ± 102.50 PPM NH_4NO_3 within 2 hours after inoculation with PV3. Pulmonary mechanical measurements (9) were made 5 days after exposure to NH_4NO_3 when the effects of the PV3 infection were greatest. The results of studies of 9 animals infected with PV3 (NH_4NO_3 unexposed) and 9 animals infected with PV3 and exposed to NH_4NO_3 are in Table 7. In addition to the usual mechanical ventilatory parameters studied, thoracic gas volume at functional residual volume (V_{TG}) was measured. No significant differences were found between the two groups for any of the mechanical or ventilatory parameters. The V_{TG} for the NH_4NO_3 exposed animals was significantly lower than for the unexposed group. Although the resistance values (R_i , R_e , R_A) tended to be higher in the exposed group, these were not significantly higher and the differences became even less when adjusted for V_{TG} . These data from these small groups of hamsters suggest that exposure to this level of NH_4NO_3 shortly after inoculation with PV3 does not markedly alter the acute course of PV3 infection. Additional studies are necessary to determine the effects of NH_4NO_3 exposure during different phases of the respiratory infection.

Lead Oxide Exposures

The PbO aerosol for these exposures was produced with a WDF (Wright Dust Feed Mechanism). The PbO used in the WDF was a dry powder Fisher Scientific Company Lead Oxide mono-yellow L-71. Animals were exposed in a nose-only plexiglass exposure chamber.

The chamber air flow was maintained at $12.5 \text{ ft}^3/\text{min}$ or about

one and a half chamber volumes per minute. Air flow was monitored with a Roots meter and a calibrated orifice. Aerosol mass was monitored by pulling samples through a 0.22 μm pore diameter filter (Millipore). Each filter was pulled using vacuum sufficient to operate the orifice at its critical flow rate. The filter holder, filter and orifice were calibrated using a wet test meter (Precision Scientific). All filter samples were collected for 45 minutes and there were two filters during each two hour exposure. The filters were analyzed using an atomic absorption spectrophotometer. Aerosol particle distribution was determined using a cascade impactor (Andersen 2000). An optical particle monitor (Royco Instruments Model 225) with Module 507 and an EAA (electrical aerosol analyzer) T51 (Model 3030) were used as continuous monitors.

A more detailed description of the exposure system, generation system and monitoring system is contained in a Toxicology Branch report, Generation of Submicron PbO aerosol with a Wright Dust Feed Mechanism (in preparation).

The effect of lead oxide exposure on the course of experimental PV3 disease was assessed using protocols similar to those in the ammonium nitrate studies. The initial experiment involved exposure of animals to lead oxide at a level of 2124 (range 1913-2371) $\mu\text{g}/\text{M}^3$ MMAD = .54 μm , σg - 1.40) of air for 2 hours, followed by inoculation with PV3 on the same day. Animals were then sacrificed on either day 3 or day 5, when maximal virus replication and pulmonary pathology, respectively, would be expected. The control consisted of animals handled identically but not exposed to lead oxide. Analysis revealed no differences in the amount of virus produced in the lung tissue (Experiment A. Table 8).

In another experimental design, groups of cotton rats were inoculated with PV3, and a portion of the animals were exposed to lead oxide aerosols at different concentrations for 2 hours on the third day of infection. Lead exposed and unexposed animals were then sacrificed on day 5 of infection for lung virus quantitation and histopathology. A few representative animals from each group were saved to be bled at 3 and at 6 weeks for PV3 antibody measurement. A series of experiments is summarized in Table 8. In Study B, in which a lead oxide exposure level of $3117 \mu\text{g}/\text{M}^3$ of air was used, the mean virus titers of lead-exposed animals were significantly higher on day 5 than among those receiving PV3 alone. The significance of this difference was supported by the serologic results. By the complement fixation method, animals receiving PV3 alone had an antibody titer of $2^{6.3}$ (geometric mean); with lead, the mean was $2^{8.75}$. The result suggests that lead-exposed animals dealt with a larger mass of viral antigen than did the other group.

In other lead level exposures (Table 8), a trend toward similar results obtained with study B were seen at the lead oxide level of $2350 \mu\text{g}/\text{M}^3$ (study C), but the differences were not statistically significant. No effect of a lower concentration of lead oxide were demonstrated (study D).

A curious result was obtained when efforts were made to reproduce study A to expand the numbers of animals. On this exposure the lead oxide level was 3,133 (range 3418-2848) $\mu\text{g}/\text{M}^3$ (MMAD = 0.62; $\sigma\text{g} - 1.77$) for 2 hours. On day 5 the mean PV3 titer in control animals was $10^{4.32}$ (n=4), while in lead-exposed animals it was $10^{3.73}$ (n=6); pathology scores were 4.75 and 5.4 in the respective

groups. Generally virus titers were lower than had been obtained previously, and no virus was found in one PV3 control. The higher pathology score in the group with the lower virus titer is highly reminiscent of the data shown in Table 1. We believe these unanticipated results were based on the fact that periodic screening of the cotton rat colony near the time of this study revealed sporadic Sendai virus seroconversions. Partial resistance of the animals to PV3 challenge and enhanced pulmonary histopathology could be explained by this problem, the importance of which is again demonstrated.

CONCLUSIONS

Since the majority of human respiratory infections have a viral etiology, experimental models of some of the major ones would be useful in assessing interactive effects with atmospheric pollutants. In this project hamsters and cotton rats were explored as models of a common human parainfluenza virus disease. The cotton rat provides the possibility for comparative studies of different etiologic agents in the same host, since they have been found susceptible not only to PV3 but to human adenovirus types 1-7, respiratory syncytial virus and Mycoplasma pneumoniae. These experimental models used in acute exposures to two particulate pollutants, ammonium nitrate and lead oxide, provided some evidence of effects on the disease. Effects included extension of pulmonary histopathology, and increase in the amount of virus in the lung tissue. The models may prove useful for chronic, or repeated acute, exposures to lower levels of the pollutants tested in these studies and other toxicants of interest. They also can be used to determine the pathogenesis of any interactive effects observed between pollutants and disease.

REFERENCES

1. Buthala, D.A. and M.G. Soret, 1964. Parainfluenza type 3 virus infection in hamsters; virologic, serologic and pathologic studies. *J. Infect. Dis.* 114:225-234.
2. Glezen, W.P and G.W. Fernald, 1976. Effect of passive antibody on parainfluenza virus type 3 pneumonia in hamsters. *Infect. Immun.* 14:212-216.
3. Barile, M.F., 1979. Mycoplasma-tissue cell culture interactions, in *The Mycoplasmas* Vol. II (J.G. Tully and R.F. Whitcomb, eds.) pp. 425-474, Academic Press, Inc., New York.
4. Hu, P.C., Kirtz, J.M., Gardner, D.E., et al., 1980. Experimental infection of the respiratory tract with *Mycoplasma pneumoniae*. *Environ Health Persp.* 35:101-106.
5. Clyde, W.A., Jr., 1971. Immunopathology of experimental *Mycoplasma pneumoniae* disease. *Infect. Immun.* 4:757-763.
6. Eaton, M.D., Meikejohn, G., van Herick, W., et al, 1942. An infectious agent from cases of atypical pneumonia apparently transmissible to cotton rats. *Science* 96:518-519.
7. Dreizin, R.S., Vyshnevetskaia, L.O., Bagdamian, E.E., et al., 1971. Experimental virus infection of cotton rats: a viral and immunofluorescent study. *Vopr. Virusol.* 16:670-676.
8. Murphy, T.F., Dubovi, E.J. and Clyde, W.A., Jr., 1981. The cotton rat as an experimental model of human parainfluenza virus type 3 disease. *Exper. Lung Res.* 2:97-109.
9. Strobe, G.L., Cox, C.L., Pimmel, R.L., and Clyde, W.A., Jr., 1980. Dynamic respiratory mechanics in intact anesthetized

hamsters. J. Appl. Physiol.: Respirat. Environ. Exercise
Physiol. 49(2): 197-203.

TABLE 1

THE EFFECTS OF PRIOR SENDAI VIRUS INFECTION ON
PV3 INOCULATION OF COTTON RATS

Exposure Groups		Results of PV3 Challenge			
First	Second	Infection (+/n)	Pneumonia (+/n)	Pathology Score*	ID ₅₀ [§] PD ₅₀ [†]
	PV3 10 ⁶	3/3	2/2	8	
Sendai 10 ⁸	10 ⁴	2/3	2/3		
	10 ²	0/3	2/3	4.7	10 ^{3.6} 10 ^{1.7}
	PV3 10 ⁶	3/3	3/3	6.3	
Sham	10 ⁴	3/3	3/3	3	≤10 ^{1.6} 10 ^{3.7}
	10 ²	2/3	0/3	0.7	

*Mean of scores 0-3+ assigned each to epithelial cell proliferation, peribronchial infiltration, endobronchial exudation, perivascular infiltration and interstitial infiltration.

[§] Dose producing infection in 50% of animals inoculated with PV3.

[†] Dose producing pneumonia in 50% of animals inoculated with PV3.

TABLE 2

CHARACTERISTICS OF BRONCHO-ALVEOLAR CELLS LAVAGED FROM
COTTON RATS INFECTED WITH P3

<u>Cells from</u>	<u>Number^a</u>	<u>Viability (%)^b</u>	<u>Cell types (%)</u>	
			<u>Macrophages</u>	<u>PMN Lymphocytes</u>
Normal (n=21)	9.50 ± 4.8	94	71	24 5
Infected, day 2 (n=6)	10.0 ± 2.1 (N.S.) ^c	93	67	28 5
4 (n=5)	14.5 ± 10.0 (N.S.)	96	62	30 8
7 (n=5)	34.0 ± 13.0 (p .005)	96	65	23 12
10 (n=5)	25.4 ± 3.4 (p .0005)	94	73	22 5

^aNumber × 10⁵, mean ± S.D.

^bTrypan blue exclusion, mean

^cStatistical comparison relative to normal animals (Student t test)

TABLE 3

COMPARISONS OF VENTILATION AND RESPIRATORY MECHANICAL
PARAMETERS FROM CONTROL AND PARAINFLUENZA VIRUS TYPE 3
INFECTED HAMSTERS*

	Control (n = 24)	P3 (n = 24)	p T
wt, g	106.3 $\pm$ 1.8	105.4 $\pm$ 1.8	0.713
V _t , ml	0.645 $\pm$ 1.8	0.601 $\pm$ 0.012	0.001
F breaths . min ⁻¹	62.6 $\pm$ 2.9	77.5 $\pm$ 2.9	0.001
V _E , ml . min ⁻¹	39.8 $\pm$ 1.5	45.5 $\pm$ 1.4	0.001
C _{dyn} , ml . cm H ₂ O ⁻¹	0.283 $\pm$ 0.021	0.203 $\pm$ 0.021	0.010
R _i , cm H ₂ O . ml ⁻¹ . s	0.340 $\pm$ 0.021	0.313 $\pm$ 0.029	0.509
R _e , cm H ₂ O . ml ⁻¹ . s	0.229 $\pm$ 0.017	0.283 $\pm$ 0.016	0.025
R _A , cm H ₂ O . ml ⁻¹ . s	0.255 $\pm$ 0.016	0.285 $\pm$ 0.016	0.181

Definition of abbreviations: P₃ = Parainfluenza virus type 3;
wt = weight; V_t = tidal volume; f = breathing frequency; V_E =
minute ventilation; C_{dyn} = dynamic compliance; R_i and R_e = inspi-
ratory and expiratory resistance; R_A = average resistance.

* Values are least square means $\pm$ 1 standard error

T p values for the differences (Student t test)

TABLE 4

EFFECTS OF NH_4NO_3 EXPOSURE¹ ON EXPERIMENTAL PARAINFLUENZA
TYPE 3 (PV3) DISEASE IN THE COTTON RAT

Exposure	<u>Observations</u>			
	Day 3		Day 6	
	Virus ²	Pathology ³	Virus	Pathology
PV3 only	4.11 (5/5)	4.3 (5/5)	2.88 (3/5)	3.0 (4/4)
PV3+ NH_4NO_3	4.02 (5/5)	2.26 (5/5)	2.8 (0/5)	4.1 (5/5)
NH_4NO_3	2.8 (0/5)	2.2 (5/5)	n.d. ⁴	n.d.

¹ $63.3 \pm .6 \text{ mg/M}^3$ (MMAD = $1.7 \text{ } \mu\text{m}$, $\sigma_g = 2.12$)

² $\text{Log}_{10} \text{TCID}_{50}/\text{gm lung}$; (no. positive/total)

³ Mean score; (no. positive/total)

⁴ n.d. = not done

TABLE 5

EFFECTS OF INTERCURRENT NH_4NO_3 ¹ EXPOSURE DURING EXPERIMENTAL
PARAINFLUENZA VIRUS TYPE 3 (PV3) DISEASE IN THE COTTON RAT

Exposure	Observation Day						
	1	3	4	5	6	7	14
Lung Pathology ² :							
PV3 only			5.0 (2/2)	5.0 (2/2)	4.0 (3/3)	3.5 (2/2)	2.75 (2.2)
PV3 + NH_4 day 3			3.4 (5/5)		5.1 (5/5)		2.0 (2/2)
PV3+ NH_4 day 5					4.8 (5.5)	3.4 (6/6)	2.67 (3/3)
NH_4 only		1.0 (1/3)	2.0 (2/2)				
Virus Titration ³ :							
PV3 only			4.13 (2/2)	3.68 (2/2)	.956 (2/4)	2.8 (0/2)	2.8 (0/2)
PV3+ NH_4 day 3			3.46 (5/6)	4.35 (2/2)	0.98 (2.7)		2.8 (0/2)
PV3+ NH_4 day 5					3.86 (6/6)	2.8 (0/4)	2.8 (0/2)
NH_4		---	---				---

¹62630 g/M³ $\pm$ 4682.85; (average $\pm$ standard deviation) MMAD = 1.6 μm ; σg = 2.10

²Mean score; (no. positive/total)

³Log₁₀ TCID₅₀/gm. lung; (no. positive/total)

TABLE 6

EFFECTS OF NH_4NO_3^1 EXPOSURE ON EXPERIMENTAL
PARAINFLUENZA VIRUS TYPE 3 DISEASE IN THE COTTON RAT

Exposure	Observation Day				
	0	1	3	6	7
Lung Pathology ² :					
PV3 only			0 (0/4)	2.25 (2/4)	2.0 (1/4)
PV3+ NH_4NO_3 day 0			2 (1/4)		
PV3+ NH_4NO_3 day 3				2.25 (2/4)	2.3 (3/4)
NH_4NO_3 only	0 (0/4)	0 (0/4)			
Virus Titration ³ :					
PV3 only			---	4.63	3.2
PV3+ NH_4NO_3 day 0			---		
PV3+ NH_4NO_3 day 3				4.97	2.8
NH_4NO_3 only	---	---			

¹0.76 mg/M³

²Mean score; (no. positive/total)

³Log₁₀ TCID₅₀/gm lung; (no. positive/total)

TABLE 7

COMPARISONS OF VENTILATORY AND RESPIRATORY MECHANICAL
PARAMETERS FROM HAMSTERS INFECTED WITH PARAINFLUENZA VIRUS

TYPE 3 WHICH WERE EITHER EXPOSED OR UNEXPOSED TO

AMMONIUM NITRATE NH_4NO_3^*

	NH_4NO_3 Unexposed (n=9)	NH_4NO_3 Exposed (n=9)
wt, g	89 ± 11	86 ± 8
V_t , ml	0.550 ± 0.043	.547 ± 0.062
F, breaths . min ⁻¹	65.2 ± 19.7	54.2 ± 8.7
V_E , ml . min ⁻¹	35.4 ± 9.9	30.9 ± 5.8
C_{dyn} , ml . cm H ₂ O ⁻¹	0.168 ± 0.075	0.200 ± 0.073
R_i , cm H ₂ O . ml ⁻¹ . s	0.378 ± 0.234	0.496 ± 0.140
R_e , cm H ₂ O . ml ⁻¹ . s	0.358 ± 0.203	0.455 ± 0.165
R_A , cm H ₂ O . ml ⁻¹ . s	0.361 ± 0.184	0.465 ± 0.135
V_{TG} , ml	2.44 ± 0.30	2.05 ± 0.26**

Definition of abbreviations: wt = weight; V_t = tidal volume; f = breathing frequency; V_E = minute ventilation; C_{dyn} = dynamic compliance; R_i and R_e = inspiratory and expiratory resistance; R_A = average resistance; V_{TG} = Thoracic gas volume at end expiration.

* 988.25 ± 162.50 $\mu\text{g}/\text{M}^3$ (average ± standard deviation)

** Significantly different from unexposed at $p < 0.01$. (Student t test)

TABLE 8

EFFECTS OF INTERCURRENT PbO_2 EXPOSURE ON EXPERIMENTAL PV3 DISEASE IN COTTON RATS

Study	PbO_2 ($\mu\text{g}/\text{M}^3$)	level (range)	MVAD (μm)	σ g	PV3 Pb-exposed	TCID ₅₀ /gm lung (n)	(log ₁₀) Control (n)	Δ Groups	P
A	2142	(1913-2371)	3.89	0.54	1.40	(10)	4.16 (6)	-0.27	n.s.
B	3117	2758-3476	0.82	1.63	4.56	(5)	3.95 (4)	0.61	.01
C	2350	2300-2400	0.73	2.10	5.90	(8)	5.27 (9)	0.63	n.s.
D	1676	1252-2101	0.89	1.67	4.82	(9)	4.94 (10)	-0.12	n.s.

* In Study A, PbO_2 exposure preceded PV3; in B, C and D pollutant followed virus inoculation.